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sufficiently large to insure a permanent value to this collection, and to warrant an attempt to make it as complete and comprehensive as may be practicable.

FREDERICK V. COVILLE,  
*Curator of Botany*

### SPECIAL ARTICLES

#### REVERSIBILITY IN ARTIFICIAL PARTHENOGENESIS

##### I

IN 1900 the writer pointed out that in *Campanularia* a highly differentiated organ like the polyp may be transformed into the less differentiated material of the stem, which in turn may form a new polyp.<sup>1</sup> Since then, reversibility of certain phenomena of differentiation has been observed by Driesch, Child, F. Lillie, Schultz and others.

The writer has repeatedly tried to reverse the phenomena of development in the egg of *Strongylocentrotus* fertilized with sperm but thus far without success. Experiments on artificial parthenogenesis, however, gave positive results.

It is difficult to cause artificial parthenogenesis in the eggs of the Californian sea urchin with hypertonic sea water. If we treat these eggs for about 2 or 2½ hours with such a solution (50 c.c. sea water + 8 c.c. 2½ *m* NaCl + CaCl<sub>2</sub> + KCl) it often happens that a certain percentage of eggs, after they have been returned to normal sea water, begin to segment regularly in 2, 4 or even 8 or 16 cells. They then stop developing and go into the condition resembling that of a resting egg. If such blastomeres are at any time fertilized with sperm they will develop into larvæ in a perfectly normal way.<sup>2</sup> These observations show incidentally that it is not the lack of the organs of cell division which prevents the unfertilized eggs from developing, since these eggs had been in possession of these organs.

The writer has shown that the induction of development in the egg is due to a combination of at least two agencies. The one causes

an alteration of the surface (which may or may not be followed by a membrane formation) and this alteration starts the development of the egg, but leaves it, in many cases at least, in a sickly condition from which it can be freed by the application of the second, corrective agency. The alteration of the surface may be caused by any of those substances or conditions which cause hemolysis: acids, bases, hydrocarbons, hypertonic and hypotonic salt solutions, foreign blood, etc. The second, curative effect may be produced by a short treatment of the egg with a hypertonic solution or by a suppression of the development of the egg for a somewhat longer period by lack of oxygen or by KCN. One method of causing artificial parthenogenesis in the eggs of *Arbacia* consists in putting them for about 20 minutes into a mixture of 50 c.c. *m*/2 (NaCl + KCl + CaCl<sub>2</sub>) + 0.3 c.c. *N*/10 NH<sub>4</sub>OH and subsequently into a neutral hypertonic solution for from 15 to 20 minutes (the figures are given for about 22° C.). A varying percentage of eggs treated this way will develop into embryos and the rest will perish very rapidly. If the eggs are treated with the alkaline solution alone without subsequent treatment with the hypertonic solution they will begin to segment, but they will perish rapidly. The alkaline treatment alone induces the change in the surface of the egg required to start the development, but this, without the corrective treatment, leads only to the first segmentations followed by a rapid disintegration.

The writer found last summer that these effects are reversible in the eggs of *Arbacia*. If, after the treatment with alkaline solution alone or with alkaline and hypertonic solution, the eggs of *Arbacia* are put for a sufficient length of time into sea water containing a certain amount of NaCN or of chloralhydrate, they go back into the resting stage and behave in appearance and reaction like unfertilized eggs. Both the NaCN and the chloralhydrate prevent the developmental processes in the egg. The suppression of these processes of development reverses the changes induced in the egg by the treatment with alkali. If after

<sup>1</sup> *Am. Jour. Physiol.*, IV., 60, 1900.

<sup>2</sup> *Arch. f. Entwicklgsmech.*, XXIII., 479, 1907;  
*Jour. Exper. Zool.*, XV., 201, 1913.

a sufficient length of time such eggs are removed from the sea water containing NaCN to normal sea water they neither segment nor disintegrate, and if sperm is added they will develop into normal blastulae. If the eggs remain only 20 minutes in the alkaline solution a very short exposure to the NaCN solution suffices. The longer the eggs remain in the alkaline solution the longer they must also remain in the cyanide solution if the effect of the alkaline solution is to be reversed. If they remain too long in the alkaline solution a subsequent treatment of the eggs with NaCN will only temporarily suppress the effects of the alkali, but as soon as they are put back into normal sea water they will disintegrate or develop. In this case the effects of alkali become irreversible.

What has been said for the effects of the alkali is also true for the effects of acid. If we cause artificial membrane formation by butyric acid in the eggs of *Arbacia* (without submitting them to the second treatment) they will begin to develop, but will disintegrate very rapidly. If they are put after the membrane formation for some hours into a cyanide solution they will go back into a resting stage. When transferred to sea water they will neither segment nor disintegrate, and when fertilized by sperm they will develop into normal larvæ.

It is therefore obvious that the induction of development in the egg of *Arbacia* by acid or by alkali is a reversible process.

## II

The question arises: Which of the two factors is reversible, the surface change (or its effect in inducing development) or the corrective factor, or both? The experiments show plainly that the first factor is reversible. In this respect the eggs of *Arbacia* differ from those of *Strongylocentrotus*. In the latter the writer succeeded in suppressing temporarily the disintegration following artificial membrane formation by the suppression of development with KCN, but the eggs when put back into normal sea water either developed or perished. There was no such reversion of the

induction to development as we find in the egg of *Arbacia*. This difference in the behavior of both kinds of eggs is possibly connected with a difference in the degree and possibly also the character of the alteration of the cortical layer under the influence of butyric acid. This is indicated externally by the difference of the membrane to which the writer had called attention in previous publications. While both types of alterations of the cortical layer induce development, in the egg of *Arbacia* this change is of a degree or character so as to be reversible, while in the egg of *Strongylocentrotus* it is irreversible as far as my present experience goes. When the eggs of *Arbacia* are exposed too long to the alkaline solution the change induced becomes also irreversible.

In the egg of *Strongylocentrotus* the corrective factor is, as the writer has recently shown, irreversible. When eggs, once treated with a hypertonic solution which does not alter them visibly and which leaves them intact, are at any time after one or two days treated with butyric acid, they will not disintegrate, but develop in the same way as if the hypertonic treatment had been applied *after* the membrane formation. I have not yet tried whether or not the same is true for the egg of *Arbacia*.

## III

It is impossible to state at present what the nature of the reversible change is. The idea has been expressed by R. Lillie that the inducement of development (membrane formation) consists in a rapid increase of permeability and that the action of the hypertonic solution is to restore a normal condition of permeability in the egg.<sup>3</sup> If this were the case, the simultaneous application of the alkaline and hypertonic solution should leave the egg wholly or nearly intact, while in fact it is just as effective as if we treat the egg first with an alkaline solution and then with a hypertonic solution. Moreover, the hypertonic solution itself induces an alteration of the surface of the egg (membrane formation) which in the terms of this hypothesis would

<sup>3</sup> Lillie, *Jour. of Morphol.*, XXII., 695, 1911.

be interpreted to mean an increase in permeability. Finally, the treatment of the egg of *purpuratus* with a hypertonic solution may precede the artificial membrane formation by one or two days. According to Lillie's hypothesis, NaCN should diminish the permeability of the egg. Direct observations by Wasteneys and myself have shown that NaCN does not influence its permeability.

The reversion of the induction of development is clearly the outcome of the suppression of the developmental changes in the egg by NaCN or by chloralhydrate. During this period of rest the cortical layer may return permanently to a condition resembling that of the normal resting egg. Since fertilization by sperm, artificial membrane formation, and destruction of the egg by cytolysis, all raise the rate of the oxidations in the egg of *purpuratus* by the same amount, the clue to the explanation of the phenomena of reversibility may possibly be found in those conditions of the cortical layer which have to do with the increase in the rate of oxidations after membrane formation.

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#### SOCIETIES AND ACADEMIES

##### BIOLOGICAL SOCIETY OF WASHINGTON

THE 414th regular meeting was held in the assembly hall of the Cosmos Club, October 18, 1913, with former President L. O. Howard in the chair and 61 persons present.

The program consisted of three communications:

#### I. *The Federal Migratory Bird Regulations and their Assistance in the Conservation of Bird Life in America*: T. S. PALMER.

The speaker outlined briefly the history of the Weeks-McLean bill, approved March 4, 1913, and of the adoption of regulations for its enforcement which have been promulgated by the Department of Agriculture under proclamation of the President dated October 1, 1913. Maps of the winter and breeding ranges of some of the species of birds affected were shown, together with another showing the division of the country into two zones. Reasons were given for the exceptions in certain states to the general closed season. In general the

beneficial effects upon the bird life of the country expected as a result of the enforcement of the federal law were pointed out.

Hugh Smith and Col. Joseph H. Acklen took part in the discussion which followed.

#### II. *The Breeding of the Loggerhead Turtle*: W. P. HAY.

The communication was accompanied by lantern slides. It was an account of observations of the habits and reproduction of the diamond-backed terrapin and the loggerhead turtle made at Beaufort, North Carolina. This place is near the northern limit of the distribution of the loggerhead turtle and the speaker was of the opinion that normally in this latitude few of the eggs of the species are left to hatch and that the young from those that may hatch all perish with the first cold weather.

#### III. *The First Year's Results in Breeding Some Bahama Shells (Cerion) on the Florida Keys*: PAUL BARTSCH.

A former communication by the speaker gave an account of the transfer of two races of *Cerion* from the Bahamas to various Florida Keys. The present paper was an account of observations of the condition of the new colonies at the end of the first year. In general they have prospered and in several localities have reproduced young.

The 515th meeting was held in the hall of the Cosmos Club, November 1, 1913, with President E. W. Nelson in the chair and about 50 members present.

Under the heading "Brief Notes and Exhibition of Specimens," C. Dwight Marsh related an observation in Montana of a noise made by a bull snake (*Pituophis sayi*) which was in close imitation of that made by a rattlesnake. The sounds were made by the respiratory organs and were observed by a number of persons.

The regular program follows.

A. D. Hopkins spoke of Depredations by Forest Insects and their Control. He gave a brief historical sketch of early insect invasions of forests and of the means adopted to combat the pests. The greater part of the paper was devoted to depredations of which the author had personal knowledge. The efficacy of modern methods was pointed out, especially the control work undertaken by the Bureau of Entomology in collaboration with the United States Forest Service. These have been generally adopted by large private holders of timber lands and much saving of valuable timber has resulted.